(TCD-BM) was transplanted in combination with untreated, fludarabine-treated, 7.5Gy γ-irradiated, or PUVA-treated splenocytes. GvL activity was studied by administering a lethal number of H2k T lymphoma cells (LBRM) or H2b myeloid lymphoma cells (C1498). Post-transplant survival of recipient groups was determined, and GvHD and GvL effects were assessed by clinical and pathological scoring. Hematopoietic chimerism and donor T cell expansion were analyzed by flow cytometric analysis of peripheral blood samples at days 30 and 60 post-BMT. In addition, the short-term in vitro survival of memory and naive donor T cell subsets was monitored after fludarabine, PUVA, or γ-irradiation. **Results:** In vitro survival of all donor T cell subsets 2 days after γ-irradiation or PUVA was minimal, while fludarabine-treated T cells demonstrated preferential survival of memory T-cells. Allogeneic splenocytes treated with fludarabine, 7.5Gy γ-irradiation, or PUVA had significantly diminished GvHD activity compared to untreated donor splenocytes, and facilitated engraftment of low-dose TCD-BM. Fludarabine-treated splenocytes (and PUVA-treated, to a lesser extent) retained GvL activity and contributed more to donor T cell engraftment compared to γ-irradiated donor splenocytes. The results are consistent with other studies suggesting that donor memory T-cells contribute to GvL activity but do not produce GvHD. **Conclusions:** Among ex vivo methods tested that inhibited GvHD activity of allogeneic lymphocytes, ex vivo treatment with fludarabine is superior to γ-irradiation or PUVA, resulting in better separation of GvHD and GvL activities in murine models of allogeneic BMT.

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REVEALING KINETICS OF CYTOKINE INDUCED KILLER CELL TRAFFICKING AND SURVIVAL IN VIVO

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Cytokine induced killer cells (CIK), which are generated from splenocytes in mice and PBMC in human by the timed addition of IFN-γ, anti-CD3 MAbs and IL-2, express both T cell and NK cell markers. CIK cells kill tumors through NKG2D mediated cytotoxicity and have in vivo activity in several murine models. CIK cells have been utilized in the clinic after both auto and allo transplant to treat or possibly reduce the risk of disease recurrence. Our goal was to explore CIK kinetics in vivo in the absence of exogenous cytokines. To this end, we transplanted luciferase-labeled murine CIK cells to compare the trafficking patterns in different transplant settings (syngeneic vs allogeneic, and myeloablative vs nonmyeloablative) and identified the survival time of CIK cells in each BMT setting by in vivo bioluminescence imaging (BLI). BLI studies showed that CIK cells were proliferated rapidly in secondary lymphoid organs such as the spleen, cervical and mesenteric lymph nodes. This observation was similar to the pattern of fresh splenocyte administration. In contrast, severe acute GVHD was not observed even in CIK dose escalation studies. CIK cell derived signals were detected more than 80 days after BMT. Moreover to clarify which lymphocyte subpopulations mainly proliferate in vivo, we also transplanted CIK cells generated from GFP positive splenocytes and sequentially analyzed tissue distribution. We confirmed that GFP+CD8+NKG2D+ cells expanded in vivo in allogeneic BMT models. In contrast, syngeneic CIK cells did not home to any specific organs and proliferated much less compared to those of allogeneic CIK cells, but GFP positive cells were detected for at least 21 days after transplantation. We demonstrated that the kinetics of CIK cell survival was different among each BMT setting and CIK cells could survive without the addition of exogenous cytokines in vivo for prolonged periods, especially in allogeneic BMT settings.

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IMPACT OF ALLOGENEIC SIBLING DONOR-DERIVED PRE-TRANSPLANTATION CD16/56+CD3+ CELLS

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The associations between the numbers of donor-derived pre-transplantation CD16/56+CD3+ cells and clinical outcome were investigated. Blood samples were obtained from 41 adult HLA-matched sibling donors on the day of transplantation. The median percentage of CD16/56+ cells recovered from the total MNCs of the donors was 8.5% (range, 0.9–27%). In addition, the median percentage of CD16/56+CD3+ cells from these populations was 3.2% (range, 0.1–10.6%). Patients who received high levels of donor CD16/56+CD3+ cells showed more favorable outcomes. The numbers of donor CD16/56+CD3+ cells were associated with the development of acute GVHD ($P = .028$) and chronic GVHD ($P = .0318$), suggest a trend towards an inverse correlation between the numbers of CD16/56+CD3+ cells and the incidence of GVHD. The numbers of donor CD16/56+CD3+ cells in association with the disease-free survival were not statistically significant ($P = .0943$). However, the higher numbers of donor CD16/56+CD3+ cells infused showed significantly lower rates of transplant-related complications (TRC) ($P = .02$). These results suggest that the levels of donor CD16/56+CD3+ cells may be an evaluable parameter to consider when performing allogeneic hematopoietic stem cell transplantation in terms of limiting the chances of TRC, including GVHD or relapse.

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SELECTIVE DOWNREGULATION OF ALLOREACTIVITY IN A HUMAN MODEL OF EXTRACORPOREAL PHOTOTHERAPY TREATMENT OF GRAFT-VERSUS-HOST DISEASE

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Graft-versus-host disease (GvHD) remains the most serious complication following haemopoietic stem cell transplantation, with an incidence of 40-60% and can be fatal in up to 50% of cases. Extracorporeal phototherapy (ECP) is a novel treatment of both acute and chronic GvHD involving psoralen and UVA (PUVA) treatment of peripheral blood cells with high reported success even in those resistant to conventional immunosuppressive treatments. ECP appears to induce selective immune suppression without increased rates of infection or disease relapse, but its mechanism of action remains poorly understood. In our department the human skin explant model for GvHD has been shown to be highly predictive of clinical GvHD and has been used to investigate the pathophysiology of the disease. The model involves sensitizing donor lymphocytes with recipient lymphocytes in vitro in a primary mixed lymphocyte reaction and then evaluating the secondary response on recipient skin biopsies by grading the graft versus host reactivity (grades I–IV) histopathologically using the Lerner grading system for GvHD. ECP only treats a small proportion of circulating mononuclear cells at each visit, but is able to downregulate GvHD through effects on untreated cells. Similarly in the skin explant model, Graft-versus-host reactivity of untreated cells was inhibited in 11 out of 19 ($P = .007$) experiments by combining the untreated cells with PUVA treated cells. Investigation of the mechanism of this downregulation of alloreactivity revealed inhibition of T cell proliferation with a dose response dependent upon the ratio of PUVA treated cells to untreated cells. Furthermore downregulation of T cell proliferation only occurred when PUVA treatment was performed on sensitized responder cells. Treatment of naive cells caused minimal or no inhibition. This selective downregulation of an alloresponse has previously been shown in mice, but this is the first demonstration of the selective action in a human system. Initial experiments have also shown that combining PUVA treated and untreated sensitized cells results in downregulation of cell-mediated cytotoxicity. This unique human model of ECP will allow investigation of cell-mediated and soluble factor changes associated with PUVA treatment of mononuclear cells and thus enable us to better understand the mechanism of action of